

WHAT IS CLAIMED IS:

1. An isolated, protein comprising an N-terminal amino acid and a C-terminal amino acid, wherein the protein is selected from the group consisting of:

(a) a protein with an N-terminal cysteine that is appended with at least one hydrophobic moiety;

(b) a protein with an N-terminal amino acid that is not a cysteine appended with at least one hydrophobic moiety; and

(c) a protein with at least one hydrophobic moiety substituted for the N-terminal amino acid.

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2. The protein of claim 1, wherein the hydrophobic moiety is a peptide comprising at least one hydrophobic amino acid.

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3. The protein of claim 1, wherein the hydrophobic moiety is a lipid.

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4. The protein of claim 1, wherein the protein further comprises a hydrophobic moiety substituted for, or appended to, the C-terminal amino acid.

5. The protein of claim 1, wherein the protein is an extracellular signaling protein.

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6. The protein of claim 1, wherein the N-terminal amino acid is a functional derivative of a cysteine.

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7. The protein of claim 1, wherein the protein is modified at both the N-terminal amino acid and the C-terminal amino acid.

8. The protein of claims 4 or 7, wherein the protein has a hydrophobic moiety substituted for, or appended to, at least one amino acid intermediate to the N-terminal and C-terminal amino acids.

9. The protein of claim 1, wherein the protein has a hydrophobic moiety substituted for, or appended to, at least one amino acid intermediate to the N-terminal and C-terminal amino acids.

10. The protein of claim 3, wherein the lipid moiety is a fatty acid selected from saturated and unsaturated fatty acids having between 2 and 24 carbon atoms.

11. The protein of claim 1, wherein the protein is a hedgehog protein obtainable from a vertebrate source.

12. The protein of claim 11, wherein the hedgehog is obtainable from a human or rat.

13. The protein of claim 11, wherein the vertebrate hedgehog is selected from the group consisting of Sonic, Indian, and Desert hedgehog.

14. The protein of claim 1, further comprising a vesicle in contact with the hydrophobic moiety.

15. The protein of claim 14, wherein the vesicle is selected from the group consisting of a cell membrane, a micelle, and a liposome.

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16. The protein of claim 11, wherein the hedgehog protein has an amino acid sequence according to any one of SEQ ID NOS: 1-4.

5 17. The protein of claim 13, wherein the hedgehog protein is missing between 1 and about 10 amino acids from the C-terminus thereof, when compared to a wild-type hedgehog protein.

18. The protein of claim 16, wherein the protein has at least 60% amino acid identity to Sonic, Indian or Desert hedgehog.

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19. An isolated, protein of the form: A-Cys-[Sp]-B- [Sp]- X, wherein

A is a hydrophobic moiety; D

Cys is a cysteine or functional equivalent thereof;

[Sp] is an optional spacer peptide sequence;

15 B is a protein comprising a plurality of amino acids and, optionally, another spacer peptide sequence; and

X is optionally another hydrophobic moiety linked to an amino acid of protein B.

20 20. The isolated protein of claim 19, wherein the isolated protein is a hedgehog protein.

21. The isolated protein of claim 20, wherein, if X is present, then it is cholesterol.

25 22. The isolated protein of claim 19, wherein protein B is modified at at least one other amino acid with at least one hydrophobic moiety.

23. The isolated protein of claim 19, wherein the A-Cys linkage is via an amino group of cysteine. D

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27. The vesicle of claim 26, wherein the vesicle is selected from the group consisting of a cell membrane, liposome and micelle.

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29. An isolated, protein having a C-terminal amino acid and an N-terminal amide group, said group formed by reacting a fatty acid thioester with an N-terminal cysteine of the protein.

30. An isolated, protein having a C-terminal amino acid and an N-terminal maleimide group, said N-terminal maleimide group formed reacting a maleimide group with the N-terminal cysteine of the protein.

31. The isolated protein of claims 28, 29 or 30, wherein the C-terminal amino acid of the protein is modified with an hydrophobic moiety.

32. The isolated protein of claim 31, wherein the protein is a hedgehog protein.

33. The isolated protein of claim 32, wherein the C-terminal hydrophobic moiety is cholesterol.

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34. A method of generating a multivalent protein complex comprising the step of linking, in the presence of a vesicle, a hydrophobic moiety to an N-terminal cysteine of a protein, or a functional equivalent of the N-terminal cysteine.

10 35. The method of claim 34, wherein the step of linking comprises linking a lipid moiety which is selected from saturated and unsaturated fatty acids having between 2 and 24 carbon atoms.

36. The method of claim 34, wherein the protein is a hedgehog protein.

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37. The method of claim 36, wherein the hedgehog is selected from the group consisting of Sonic, Indian and Desert hedgehog.

20 38. The method of claim 36, wherein the hedgehog has an amino acid sequence according to any one of SEQ ID NOS: 1-4.

39. The method of claim 34, wherein the step of linking comprises linking with a vesicle selected from the group consisting of a cell membrane, liposome and micelle.

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40. A method for modifying a physico-chemical property of a protein, comprising introducing at least one hydrophobic moiety to an N-terminal cysteine of the protein or to a functional equivalent of the N-terminal cysteine.

41. The method of claim 40, further comprising contacting the hydrophobic moiety with a vesicle.

5 42. The method of claim 40, wherein the hydrophobic moiety is either a lipid moiety selected from saturated and an unsaturated fatty acids having between 2 and 24 carbon atoms or is a hydrophobic protein.

43. The method of claim 40, wherein the protein is a hedgehog protein.

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44. The method of claim 43, wherein the hedgehog protein is selected from the group consisting of Sonic, Indian and Desert hedgehog.

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45. The method of claim 43, wherein the hedgehog has an amino acid sequence according to any one of SEQ ID NOS: 1-4.

46. The method of claim 41, wherein the step of contacting comprises contacting with a vesicle selected from the group consisting of a cell membrane, liposome and micelle.

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47. A protein complex, produced by the method of claim 34.

48. A modified protein, produced by the method of claim 40.

25 49. The complex of claim 47, wherein the protein is selected from the group consisting of gelsolin; an interferon, an interleukin, tumor necrosis factor, monocyte colony stimulating factor, granulocyte colony stimulating factor, granulocyte

macrophage colony stimulating factor, erythropoietin, platelet derived growth factor, growth hormone and insulin.

50. A method for modifying a protein having a biological activity and containing an N-terminal cysteine, comprising reacting the N-terminal cysteine with a fatty acid thioester to form an amide, wherein such modification enhances the protein's biological activity.

51. The method of claim 50, wherein the protein is a hedgehog protein.

52. The method of claim 51, wherein the hedgehog protein is selected from the group consisting of Sonic, Indian, Desert hedgehog, and functional variants thereof.

53. A method for modifying a protein having a biological activity and containing an N-terminal cysteine, comprising reacting the N-terminal cysteine with a maleimide group, wherein such modification enhances the protein's biological activity.

54. The method of claim 53, wherein the protein is a hedgehog protein.

55. The method of claim 54, wherein the hedgehog protein is selected from the group consisting of Sonic, Indian, Desert hedgehog, and functional variants thereof.

56. A method for modifying a protein having a biological activity comprising appending an hydrophobic peptide to the protein.

57. The method of claim ~~56~~, wherein the hydrophobic peptide is appended to an amino acid of the protein selected from the group consisting of the N-terminal amino acid, the C-terminal ~~amino acid~~, an amino acid intermediate between the N-terminal amino acid and the C-terminal amino acid, and combinations of the foregoing.
58. The method of claim ~~69~~, wherein the protein is a hedgehog protein.
59. The method of claim ~~71~~, wherein the hedgehog protein is selected from the group consisting of ~~Sonic~~, Indian and Desert hedgehog.
60. A therapeutic use of the protein of any of claims 1 or 20, comprising administering the protein to a subject.
61. A method of treating a neurological disorder in a patient comprising administering to the patient a protein of any of claims 1 or 20.
62. The protein of claim ~~1~~, wherein the protein is an extracellular signaling protein.
63. The method of claim ~~57~~, wherein the step of appending comprises replacing at least the N-terminal amino acid of the protein with at least one hydrophobic amino acid.
64. The method of claim ~~63~~, wherein the at least one hydrophobic amino acid is a plurality of isoleucine residues.
65. The method of claim 63, further comprising chemically modifying at least one of the isoleucine residues.



66. An isolated, protein having a C-terminal amino acid and an N-terminal acetamide group, said group formed by reacting a substituted acetamide with an N-terminal cysteine of the protein.

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67. An isolated, protein having a C-terminal amino acid and an N-terminal thiomorpholine group, said group formed by reacting a haloketone group with an N-terminal cysteine of the protein.

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68. A method for modifying a protein having a biological activity and containing an N-terminal cysteine, comprising reacting the N-terminal cysteine with a substituted acetamide group, wherein such modification enhances the protein's biological activity.

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69. The method of claim 68, wherein the protein is a hedgehog protein.

70. The method of claim 69, wherein the hedgehog protein is selected from the group consisting of Sonic, Indian, Desert hedgehog, and functional variants thereof.

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71. A method for modifying a protein having a biological activity and containing an N-terminal cysteine, comprising reacting the N-terminal cysteine with a haloketone group, wherein such modification enhances the protein's biological activity.

72. The method of claim 71, wherein the protein is a hedgehog protein.

73. The method of claim 72, wherein the hedgehog protein is selected from the group consisting of Sonic, Indian, Desert hedgehog, and functional variants thereof.

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- 5 74. A hedgehog polypeptide modified with one or more lipophilic moieties with the proviso that, in the instance wherein the hedgehog polypeptide is the mature N-terminal proteolytic fragment of a hedgehog protein, the lipophilic moiety is other than a sterol at the C-terminal residue.
75. A hedgehog polypeptide modified with one or more lipophilic moieties at internal amino acid residues.
- 10 76. A hedgehog polypeptide modified with one or more lipophilic aromatic hydrocarbons.
77. The hedgehog polypeptide of any of claims 74-76 which polypeptide is provided as a purified protein preparation.
- 15 78. The hedgehog polypeptide of any of claims 74-76 which polypeptide is provided as a pharmaceutical preparation.
- 20 79. The hedgehog polypeptide of claim 74 or 75, wherein the lipophilic moieties are selected from the group consisting of fatty acids, lipids, esters, alcohols, cage structures, and aromatic hydrocarbons.
- 25 80. The hedgehog polypeptide of claim 76 or 79, wherein the aromatic hydrocarbon is selected from the group consisting of benzene, perylene, phenanthrene, anthracene, naphthalene, pyrene, chrysene, and naphthacene.
81. The hedgehog polypeptide of claim 80, wherein the aromatic hydrocarbon is a pyrene.
- 30 82. The hedgehog polypeptide of claim 74 or 75, wherein the lipophilic moieties are selected from the group consisting of isoprenoids, terpenes and polyalicyclic hydrocarbons.
- 35 83. The hedgehog polypeptide of claim 82, wherein the lipophilic moieties are selected from the group consisting of adamantanes, buckminsterfullerenes, vitamins, polyethylene glycol, oligoethylene glycol, (C1-C18)-alkyl phosphate diesters, -O-CH<sub>2</sub>-CH(OH)-O-(C12-C18)-alkyl.
- 40 84. The hedgehog polypeptide of claim 83, wherein the lipophilic moieties are selected from the group consisting of 1- or 2-adamantylacetyl, 3-methyladamant-1-ylacetyl, 3-methyl-3-bromo-1-adamantylacetyl, 1-decalinacetyl, camphoracetyl, camphaneacetyl, noradamantylacetyl, norbornaneacetyl, bicyclo[2.2.2.]-oct-5-

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85. The hedgehog polypeptide of any of claims 74-76, wherein the lipophilic moiety or moieties potentiate the biological activity of the polypeptide relative to the modified hedgehog polypeptide.
- 10 86. A method for altering the growth state of a cell responsive to hedgehog signaling, comprising contacting the cell with a lipophilic-modified hedgehog polypeptide of any of claims 74-76.

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**Abstract**